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ThDP-Dependent Enzymes as Catalytic Tools for the Asymmetric Benzoin-Type Reaction

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Author Comments:	Ferrara, February 29th 2016 Dear Editor, Please find enclosed our manuscript entitled "ThDP-Dependent Enzymes as Catalytic Tools for the Asymmetric Benzoin-Type Reaction" that, in response to your kind invitation (e-mail of June 10th 2015), we wish to submit for publication in the European Journal of Organic Chemistry as a Microreview. We apologize for the delay and we thank you for the additional time you gave us. As already mentioned in our e-mail of January 18th 2016, the extra time was necessary to complete a study entitled "(S)-Selectivity in Phenylacetyl Carbinols Synthesis Using the Wild-Type Enzyme Acetoin:Dichlorophenolindophenol Oxidoreductase from Bacillus licheniformis", which we are going to submit to Advanced Synthesis and Catalysis in a few weeks. As we consider the results of this study of some relevance, we have discussed them in the present microreview. Hence, we are also sending you a draft of the manuscript on phenylacetyl carbinols as an attached file. In the present microreview we have tried to give an overview of the different types of benzoin reactions catalyzed by thiamine diphosphate (ThDP)-dependent enzymes according to the classification of Table 1 and we hope to have given a clear guide to the reader for accessing the class of alpha-hydroxyketone products with the desired stereochemistry. We are looking forward to hearing from you at your earliest convenience. With best regards, Pier Paolo Giovannini

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ThDP-Dependent Enzymes as Catalytic Tools for the Asymmetric Benzoin-Type Reaction

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Dedication ((optional))

Abstract: Benzoin-type reactions allow to generate α-hydroxyketones through the (formal) carboligation of two aldehyde reactants. The synthetic relevance of the products and the wide diffusion of the α-hydroxyketone functionality in bioactive natural compounds are motive for the intensive research efforts spent to develop ever more efficient and selective catalysts for this class of reactions. As for many other issues, also in this case the solution developed by Nature, that is the utilization of thiamine diphosphate (ThDP)-dependent enzymes, allows to achieve levels of chemo- and stereoselectivity nowadays unattainable by biomimetic organocatalysts. Herein, we present an overview of the structural diversity of the α-hydroxyketone motif achievable through ThDP-dependent enzyme catalysis. Details on the structure-activity relationship and on the rational mutagenesis approaches for improving the catalytic performances of wild-type enzymes will be also illustrated.

1. Introduction

Modern organic synthesis is today oriented to the development of sustainable methodologies that, according to the green chemistry principles,[1] have to be as more efficient and productive as possible, prevent waste formation and avoid the use of hazardous substances and procedures. Under this perspective, the use of enzymes as catalysts can be the winning move for many organic reactions, because they are applied under mild conditions that lower the energy requirements and the level of hazard of the processes and, in virtue of the high chemo-, regio-, and stereoselectivity typical of enzymes, few by-products are formed and protection-deprotection steps can be avoided.

Benzoin-type reactions, which are important tools for the preparation of α-hydroxyketones, represent a very promising field of application for enzyme catalysis. This type of reactions has deep roots in the history of synthetic organic chemistry since the homo-coupling of benzaldehyde catalyzed by cyanide, known as “benzoin condensation”, was firstly reported almost two centuries ago.[2] Afterwards, numerous studies contributed to expand the synthetic scope of this type of reactions; further breakthroughs were the introduction of thiazolium salts as pre-catalysts[3] and the rationalization of their catalytic mechanism.[4] In 1958, Breslow indicated the carbanion generated by the ionization of the C2 of the thiazolium ring as the catalytic active species equivalent to cyanide (structure **I** in Scheme 1). The nucleophilic attack of this species (ylide or carbene) to the carbonyl group of an aldehyde followed by the proton transfer of the former aldehydic proton leads to an enamine intermediate commonly known as Breslow intermediate (structure **III** with X = H). In this species, the electrophilicity of the aldehyde substrate is inverted. Transformations of this type are named umpolung reactions[5] and the Breslow intermediate can be considered as an acyl anion equivalent.[6] Benzoin-type reactions in the presence of azolium salts, which are precursors of active *N*-heterocyclic carbene (NHC) catalysts,[7] allow to prepare in simple, safe and inexpensive way variously substituted α-hydroxyketones, whose high synthetic value as building blocks for the synthesis of many important organic compounds and bioactive molecules is well known.[8] This is the motive for the impressive number of (NHC)-catalyzed benzoin-type reactions that have been developed in the last decades.[9] Unfortunately, in spite of their high efficiency, these reactions are rarely chemoselective[10] and highly enantioselctive.[11] The selectivity limitations of the organocatalyzed reactions made attractive the family of thiamine diphosphate (ThDP)-dependent enzymes, whose catalytic mechanism represents a natural version of the NHC-catalyzed benzoin reaction.

The structure of thiamine was known from the 1930s as well as the essential role of the cofactor ThDP for the activity of the enzyme pyruvate decarboxylase (PDC).[12] Nevertheless, the mechanism through which the decarboxylation takes place was understood only twenty years later thanks to the Breslow’s studies.

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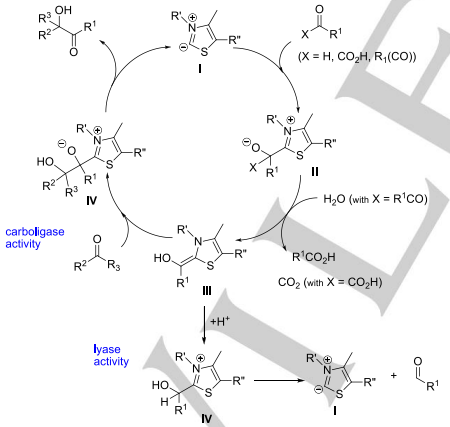
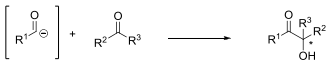


Scheme 1. ThDP-dependent enzyme-catalyzed benzoin and benzoin-type reactions.

Although the massive exploitation of enzymes in synthetic organic chemistry started from the 1980s, the first ThDP-dependent enzyme-catalyzed asymmetric syntheses of α-hydroxyketones on preparative scale was reported in 1999. In this year two independent studies, quite curiously published on the same issue of the journal, described the use of benzoylformate decarboxylase (BFD) for the preparation of various (*R*)-benzoins[13] and the phenylpyruvate decarboxylase (PhPDC)-catalyzed synthesis of (*R*)-3-hydroxy-1-phenyl-2-butanone.[14]

The general catalytic mechanism of the ThDP-enzymatic 1,2-addition of an aldehyde (X = H) to a carbonyl acceptor is depicted in Scheme 1. As shown, different donors can also afford the same acyl anion equivalent **III** (activated aldehyde), namely α-diketones (X = $R^1C(O)$) and ketoacids (X = C(O)OH). The intermediate **II** generated by the attack of the ThDP ylide **I** undergoes rearrangements, which depend on the structure of the donor substrate. Very often, it happens that the catalytic mechanisms exploited for the desired synthetic applications rely on side-reactivities not related to the physiological roles of the ThDP-dependent enzymes. In fact, many of the enzymatic carboligation reactions discussed in the present microreview are catalyzed by lyases, whose natural catalytic cycle proceeds through the protonation of the Breslow intermediate **III** leading to the release of the aldehyde corresponding to the donor acyl group by cleavage of the intermediate **V** (Scheme 1). Furthermore, in many cases, more than one enzyme/substrate pair can be utilized to produce the same target compound. For these reasons, we have considered more useful to organize this microreview in sections describing a particular combination of reagents, instead of following a classification based on the type of biocatalyst employed. In addition, a few words have to be spent on the terms "benzoin-" and "acyloin condensation" that are routinely used to indicate the umpolung addition of aromatic and aliphatic aldehydes, respectively. We believe it is more appropriate to use the terms listed in Table 1 to name all the different syntheses of α-hydroxyketones catalyzed by ThDP-dependent enzymes. Indeed, while the term "benzoin condensation" unequivocally identifies the homo-coupling of benzaldehydes to give benzoins, the term "acyloin condensation" is also used to indicate the synthesis of symmetrical α-hydroxy ketones via the reductive condensation of esters in the presence of sodium.[15]

Table 1. ThDP-dependent enzyme-catalyzed benzoin and benzoin-type reactions.

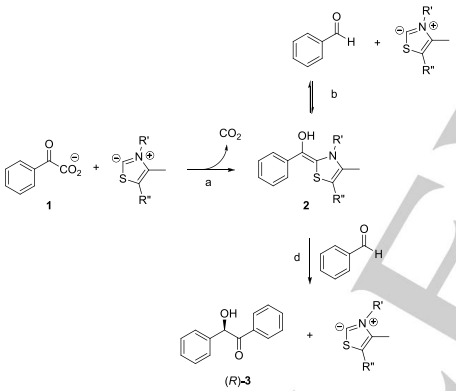


R^1	R^2	R^1, R^2	R^3	reaction
Aryl	Aryl	$R^1 = R^2$	H	benzoin
Aryl	Aryl	$R^1 \neq R^2$	H	cross-benzoin
Alkyl	Alkyl	$R^1 = R^2$	H	aliphatic benzoin-type
Alkyl	Alkyl	$R^1 \neq R^2$	H	aliphatic cross-benzoin-type
Aryl	Alkyl		H	aromatic-aliphatic cross-benzoin-type
Alkyl	Aryl		H	aliphatic-aromatic cross-benzoin-type
Alkyl[a]	Alkyl[a]		Alkyl[a]	aldehyde-ketone cross-benzoin type

[a] All the enzymatic aldehyde-ketone cross-benzoin-type reactions reported so far showed these features.

2. Benzoin reaction

Three ThDP-dependent enzymes have been used to catalyze the homo-coupling of benzaldehyde on preparative scale, namely the benzoylformate decarboxylase from *Pseudomonas putida* (*Pp*BFD), the benzaldehyde lyase from *Pseudomonas fluorescens* Biovar I (*Pf*BAL), and the mutant variant of the pyruvate decarboxylase from *Acetobacter pasteurianus Ap*PDC-Glu469Gly/Thr384Gly/Ile468Ala/Trp543Phe. The BFD plays its natural role in the degradation of aromatic compounds of the mandelate catabolism. It has been found in bacteria of *Pseudomonas* and *Alcaligenes* genus, where it catalyzes the non-oxidative decarboxylation of benzoylformate **1** (Scheme 2, steps a + b).[16] At the same time, an "out of pathway" carboligation activity takes place as demonstrated by the presence in the reaction mixture of trace amounts of (*R*)-benzoin ((*R*)-**3**).[17] The formation of **3** indicates that the life-time of the active aldehyde intermediate **2** is long enough to allow its attack to the free benzaldehyde (steps a + d). Thanks to the reversibility of step b, **3** can be formed also starting from benzaldehyde only (steps reverse b + d).[18]



Scheme 2. *Pp*BFD catalyzed reactions: non-oxidative decarboxylation of benzoylformate **1** (a+b); homo-coupling of benzaldehyde (a+d or reverse b+d).

In 1999 the *Pp*BFD-catalyzed benzoin reaction was applied to the enantioselective synthesis of variously substituted (*R*)-benzoins.[13] The results of this work (Table 2, columns 3 and 4) show that (*R*)-benzoin derivatives are formed with a high optical purity (ee 94->99%) and that *ortho*-substituted benzaldehydes, with the exception of the 2-fluoro derivative, are very poor substrates for *Pp*BFD.

Furthermore, the use of DMSO as co-solvent was demonstrated to be beneficial allowing to significantly increase the yields of benzoins (from 20 to 70%). Another enzyme successfully used for the highly enantioselective synthesis of (*R*)-benzoins is *Pf*BAL.[19] This enzyme catalyzes the ThDP-dependent cleavage of benzoin allowing the bacterium to grow in the presence of this α-hydroxyketone as the sole carbon source.[20]

Observing that the cleavage of benzoin was *R*-specific and did not reach the completion, the existence of an equilibrium between the cleavage and formation of (*R*)-benzoin **3** was postulated. The hypothesis was confirmed by the accumulation of **3** when benzaldehyde was added to a buffered aqueous solution containing *pf*BAL and a catalytic amount of ThDP and Mg^{2+}.[19a]

As observed for *Pp*BFD, also in the case of *Pf*BAL-catalyzed benzoin reactions the addition of DMSO (20%, v/v) allowed an almost quantitative conversion of benzaldehyde to enantiomerically pure (*R*)-benzoin **3** (ee > 99%). The substrate scope of *Pf*BAL catalysis was carefully investigated and a broad range of aromatic aldehydes, displaying electron-withdrawing and electron-donating groups were converted to the corresponding (*R*)-benzoins with enantioselectivities (ees) ranging from 91 to 99% (Table 2, columns 5 and 6).[19b]

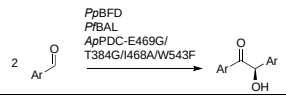
It is worthy of note that in contrast with *Pp*BFD, *Pf*BAL shows a good activity also with *ortho*-substituted benzaldehydes as substrates. The *Pf*BAL-catalyzed benzoin condensation was also subjected to medium engineering studies.[21] The enzyme entrapped in polyvinyl alcohol and suspended in hexane showed a three-fold increased productivity compared to the reaction performed in buffer with 20% DMSO and, thanks to the hydrophobic system, the product range was expanded with some highly hydrophobic benzoins.[22]

*Pf*BAL has also been employed in deep-eutectic-solvent-buffer mixtures, producing (*R*)-benzoin and (*R*)-furoin.[23] Furthermore, the (*R*)-benzoin synthesis has been also revisited under heterogeneous conditions both in a batch reactor with the enzyme immobilized on a metal-chelate epoxy support[24] and in gas phase with a continuous plug flow reactor; in this study the enzyme was deposited on a porous support.[25]

Since wild-type (wt) ThDP-dependent enzymes suited for the synthesis of (*S*)-benzoins are not known, this issue has been principally addressed through the *Pf*BAL-catalyzed kinetic resolution of the racemic precursors.[19,26] Only recently an engineered *Ap*PDC able to catalyze (*S*)-specific benzoin reactions has been developed.[27] The reverse stereoselectivity of the mutant with respect to the wt enzyme was demonstrated for the condensation of acetaldehyde and benzaldehyde that afforded (*S*)-phenylacetylcarbinol **4**.

The substitution of Glu469 with Gly opened an additional space in the acceptor binding site, the so called "S-pocket", and made possible the reciprocal antiparallel orientation of the donor and acceptor side chains during the nucleophilic attack. As a result (*S*)-**3** was formed in place of the (*R*) enantiomer.[28]

Table 2. Product range of benzoin reactions catalyzed by *Pp*BFD-, *Pf*BFD-, and ApPDC-Glu469Gly/Thr387Gly/Ile468Ala/Trp534Phe.



Entry	Aryl	*Pp*BFD		*Pf*BAL		*Ap*PDC-E469G/T384G/I468A/W543F	
		Yield (%)	Ee (%)	Yield (%)	Ee (%)	Yield (%)	Ee (%)
1	C_6H_5	70	>99 (*R*)	96	>99 (*R*)	66	98 (*S*)
2	2-FC_6H_5	68	>99 (*R*)	68	96 (*R*)	<5a	21 (*R*)
3	2-ClC_6H_5	-	-	80	97 (*R*)	n.c.	n.d.
4	2-BrC_6H_5	<2	n.d.	90	>99 (*R*)	-	-
5	2-$MeOC_6H_5$	<2	n.d.	87	>99 (*R*)	n.c.	n.d.
6	3-FC_6H_5	-	-	80	97 (*R*)	36a	93 (*S*)
7	3-ClC_6H_5	-	-	94	>99 (*R*)	72	>99 (*S*)
8	3-BrC_6H_5	-	-	94	>99 (*R*)	30a	>99 (*S*)
9	3-IC_6H_5	-	-	-	-	11a	>99 (*S*)
10	3-$MeOC_6H_5$	18	>99 (*R*)	93	>99 (*R*)	61	>99 (*S*)
11	3-HOC_6H_5			84	n.d.	-	-
12	4-FC_6H_5	25	>99 (*R*)	89	>99 (*R*)	<5a	85 (*S*)
13	4-ClC_6H_5	17	>99 (*R*)	95	>99 (*R*)	n.c.	n.d.
14	4-BrC_6H_5	13	>99 (*R*)	83	>99 (*R*)	-	-
15	4-$MeOC_6H_5$	12	>99 (*R*)	95	>99 (*R*)	n.c.	n.d.
16	4-MeC_6H_5	69	>99 (*R*)	94	>99 (*R*)	-	-
17	2-furyl	62	94 (*R*)	88	92 (*R*)	-	-
18	5-Me-2-furyl	50	96 (*R*)	-	-	-	-
19	2-thiophenyl	65	95 (*R*)	-	-	-	-
20	2-Pyr.	70	94 (*R*)	-	-	-	-
21	2,4-$F_2C_6H_3$	-	-	87	>99 (R)	-	-
22	2-naphthalenyl	-	-	98	>99 (R)	-	-

n.d. (not detected); n.c. (no conversion)

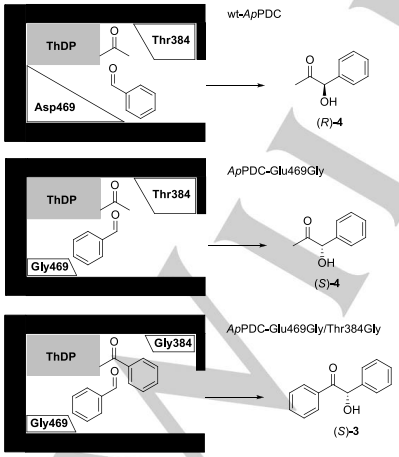


Figure 1. Schematic representation of the active site architecture of the wt-, Glu469Gly-, and Glu469Gly/Thr384Gly variants of *Ap*PDC.

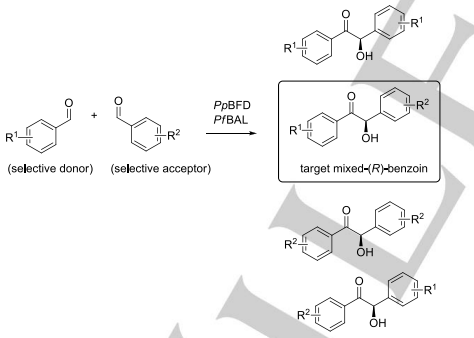
Nevertheless, because of the constrained donor binding site, benzaldehyde could not be used as donor by the *Ap*PDC-Glu469Gly mutant. This drawback was overcome by introducing a further mutation suggested by comparative studies between numerous structures and sequences of homologues PDC and BAL that allowed to recognize the larger donor binding site of BAL, which accepts aromatic aldehyde donors, as the structural base accounting for the different chemoselectivity of the two enzymes. The Thr384 was identified as the main responsible for the reduced size of the donor binding site in PDC. As a consequence, the mutation of this residue with glycine afforded the A*p*PDC-Glu469Gly/Thr384Gly variant, which was able to catalyze the (*S*)-selective benzoin reaction with good efficiency (52% conversion and 59% ee). The moderate enantioselectivity was then enhanced further remodeling the acceptor binding site by mutation of the Ile468 and Trp534, which are two residues that contribute to stabilize the parallel orientation of the aromatic acceptor (*R*-pathway). Their exchange afforded the highly (*S*)-specific four-point mutant ApPDC-Glu469Gly/Thr384Gly/Ile468Ala/Trp534Phe, which gave (*S*)-benzoin ((*S*)-**3**) in 66% yield and 98% ee and also other

benzoin analogues, especially the meta-substituted ones, with very high ees (Table 2, columns 7 and 8).[27]

3. Cross-benzoin reactions

The non-enzymatic version of this type of reaction was historically performed using the cyanide anion as the catalyst. The detected selectivity was attributed to the different reactivity that variously substituted benzaldehydes displayed with the cyanide catalyst.[29] This donor-acceptor concept inspired the authors of the unique example of enzymatic cross-benzoin reaction reported so far.[26b] The very low reactivity as donor shown by 2-chloro, 2-methoxy and 2-methylbenzaldehydes in *Pp*BFD-catalyzed carboligation reactions with acetaldehyde[18] suggested the use of such compounds as selective acceptors in cross-benzoin reactions with benzaldehyde as donor and *Pp*BFD as catalyst. Indeed, the desired mixed benzoins were obtained in the cases of 2-chloro and 2-methyl benzaldehyde although with a moderate selectivity (data not shown in Table 3). A comparable trend was also observed using *Pf*BAL as catalyst, which additionally accepted 2-substituted di-, tri, and pentafluorinated benzaldehydes as acceptors. Once identified the pool of selective acceptors, an accurate screening of the potential donors was performed. The screening, conducted using 2-Cl-benzaldehyde as selective acceptor and both the *Pp*BFD and *Pf*BAL biocatalysts, allowed to identify a number of donor/acceptor/enzyme combinations suited for the highly selective asymmetric synthesis of (*R*)-mixed benzoins.

Table 3. Chemoselective synthesis of mixed (*R*)-benzoins.



R^1	R^2	Enzyme	Conv. (%)	Select.[a] (%)	Ee (%)
3-CN	2-Cl	BFD-H281A	>99	>99	90
4-Br	2-Cl	BFD-H281A	90	95	95
4-CF_3	2-Cl	BFD-H281A	75	>99	93
3,4,5-$(MeO)_3$	2-Cl	BAL	82	97	>99
3,5-$(MeO)_2$	2-Cl	BAL	>99	95	>99
3,5-$(MeO)_2$	2,6-F_2	BAL	>99	96	97[b]
4-Br	2,3,5-F_3	BAL	>99	90	62[b]

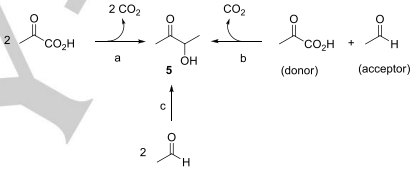
[a] Selectivity is defined as the percent ratio of product in relation to the sum of all benzoins obtained. [b] From supporting information of Ref. 15.

Table 3 reports only the results of reactions with selectivity >90%, but numerous further mixed benzoins have been asymmetrically synthesized by this approach with selectivity ranging from 60 to 90%. Finally, it has to be underscored that the *Pp*BFD used in this study was the mutant variant *Pp*BFD-His281Ala, whose improved carboligation activity was obtained by reducing the protonation of the enamine intermediate (**III** in Scheme 1), which is promoted in the wt enzyme by the His281.[30]

4. Aliphatic benzoin-type reactions

This section is dedicated to the ThDP-dependent enzyme-catalyzed homo-coupling of aliphatic aldehydes affording the corresponding symmetrically substituted acyloins. Acetoin (3-hydroxybutane-2-one) **5** is the simplest product obtainable through this approach. Its enzymatic formation has been extensively studied and used as a probe reaction to investigate the catalytic mechanisms of various ThDP-dependent enzymes.

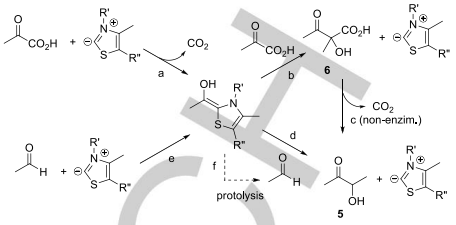
Table 4. Synthesis of acetoin mediated by ThDP-dependent enzymes.



Entry	Route	Enzyme	Acetoin **5** ee (%)	Ref.
1	a	ScPDC	46-53 (*R*)	31
2	b	ScPDC	46-50 (*R*)	31
3	c	ScPDC	44 (*R*)	31
4	a	ZmPDC	23-29 (*S*)	31
5	b	ZmPDC	28-29 (*S*)	31b,31a
6	c	ZmPDC	25 (*S*)	31
7	a	CDH	93 (*S*)	32
8	b	CDH	92 (*S*)	32
9	c	CDH	95 (*S*)	32
10	a	SucA	8 (*R*)	34
11	c	SucA	90 (*R*)	34
12	a	YerE	4 (*S*)	32,35
13	a	PigD	70 (*S*)	36
14	a	MenD	*rac.*	37
15	a	ZmPDC-Glu473Gln	33 (*R*)	31a
16	a	ZmPDC-Glu473Phe	80 (*R*)	24
17	c	ZmPDC-Glu473Phe	91 (*R*)	24
18	a	ZmPDC-Glu473Ala	98 (*S*)	24
19	c	PfBAL	40 (*R*)	25
20	c	PpBFD	34 (*R*)	25

Actually, acetoin **5** can be prepared by reactions involving three different donor-acceptor combinations, namely the homo-coupling of pyruvate (route a), the cross-coupling of

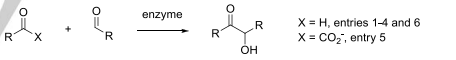
pyruvate (donor) and acetaldehyde (acceptor; route b), and the homo-coupling of acetaldehyde (route c). Table 4 summarizes the results of the enzymatic syntheses of acetoin catalyzed by some of the most extensively studied ThDP-dependent enzymes.The PDCs from *S. cerevisiae* and *Z. mobilis* (*Sc*PDC, *Zm*PDC)[31] and the cyclohexane-1,2-dione hydrolase from *Azoarcus sp*. (CDH)[32] catalyze the formation of acetoin through all the three possible routes (Table 4, entry 1-9). Accordingly, these enzymes can use pyruvate and acetaldehyde both as donor and acceptor. It is worthy of note that, independently from the different stereoselectivity shown by the three enzymes, the ee of the acetoin produced by each enzyme did not depend by the synthetic pathway followed. Interestingly, this is not true for SucA, the E1 component of the α-ketoglutarate dehydrogenase complex from *E. coli* K12,[33] which affords acetoin with high (90% *R*) or very low (8% *R*) ee starting from pyruvate or acetaldehyde, respectively (Table 4, entries 10 and 11).[34] For what concerns the enzymes YerE,[32,35] PigD,[36] and MenD[37] only data relative to the production of acetoin from pyruvate (route a) are available (Table 4, entry 12-14). As reported for SucA, also YerE and MenD convert pyruvate to acetoin in a non-stereoselective way (4% ee for YerE and racemic for MenD). By contrast, the product afforded by PigD was optically active (ee 70% *S*). The low ees obtained with SucA, YerE and MenD have been explained demonstrating (NMR experiments with ^{13}C-labeled pyruvate)[34b] that in these cases acetoin is formed by the non-enzymatic decarboxylation of acetolactate **6** that is the real product of the pyruvate homo-coupling (Scheme 4, route a + b + c). On the other hand, PDC, CDH and PigD afforded enantioenriched acetoin because in these cases the activated aldehyde attacks the free acetaldehyde, which is formed by protolysis of the same enamine intermediate (route a + f + d). This acetolactate-independent acetoin formation has been experimentally demonstrated for both the wild type and the Glu473Gln variant of *Zm*PDC[31a] as well as for CHD.[32] The behavior of SucA, which affords almost racemic (8% (*R*)) or enantioenriched acetoin (ee 90% (*R*)) by pyruvate or acetaldehyde homo-coupling (route e + d), respectively, is representative of the two alternative mechanisms. The *Zm*PDC-Glu473Gln variant was designed to increase the carboligation activity of the native enzyme since it was demonstrated for Glu473 a role in the protonation of the enamine intermediate (**III** in Scheme 1). Noteworthy, in addition to the expected increase of the C-C bond forming activity, this variant also showed reverse enantioselectivity (Table 4, entry 15).[31a] This result allowed to recognize Glu473 as a hot-spot for rational single-point mutagenesis approaches for the fine tuning and switching of the *Zm*PDC enantioselectivity.[38] Actually, the substitution of such residue with the bulky Phe (Table 4, entry 16 and 17) afforded an even more (*R*)-selective enzyme (ee 80% and 91 starting from pyruvate and acetaldehyde, respectively) compared to the mutant with Gln in the same position (ee 33%). In contrast, substitution of Glu473 with the smaller Ala (Table 4, entry 17) gave a variant highly (*S*)-selective (ee 98% starting from pyruvate).



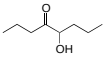
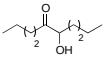
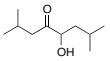
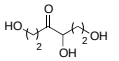
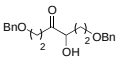
Scheme 4. Pyruvate homo-coupling through acetolactate **6** and alternative pathways.

Symmetrically substituted acyloins with more complex structures have been synthesized using various ThDP-dependent enzymes and donor-acceptor combinations. Table 5 summarizes the principal results obtained in this field. The enzymatic self-condensations of C3-C5 aliphatic aldehydes has been explored using *Pf*BAL and *Pp*BFD (entries 1-4).[39] These two enzymes gave very similar results in terms of both conversion or enantioselectivity. Apart from the reaction with propanal/PfBAL that afforded the expected acyloin with (*S*)-configuration (ee 60%), all the other substrates were converted into the corresponding products with (*R*)-configuration (ee ranging from 60 to 89%). The addition of 2-propanol as co-solvent (20% V/V) allowed to increase the *Pf*BAL enantioselectivity in the reactions with butanal (ee from 50% to 80%) and pentanal (ee from 30% to 60%) as the substrates (entries 2 and 3).

Table 5. Enzymatic aliphatic benzoin-type reactions.



(donor) (acceptor) **7**

Entry	Product **7**	Enzyme	Yield; Ee (%)	Ref.
1		*Pf*BAL	>90; 60 (*S*)	25
		*Pp*BFD	>90; 63 (*R*)	25
2		*Pf*BAL	>90; 80[a] (*R*)	25
		*Pp*BFD	>90; 80 (*R*)	25
3		*Pf*BAL	>90; 60[a] (*R*)	25
		*Pp*BFD	>90; 65(*R*)	25
4		*Pf*BAL	>90; 89 (*R*)	25
		*Pp*BFD	60; 85(*R*)	25
		KdcA	-; 30-47 (*S*)	26
5		EcTK	60; 95 (*S*)	40
		*Sc*PDC	n.d.; n.d. (*R*)	41
		PDHc-E1	n.d.; n.d. (*S*)	41
6		*Pf*BAL	91; 95 (*R*)	42

[a] With 20 % of 2-propanol as co-solvent. n.d. (not detected).

While isovaleraldehyde was efficiently converted by *Pf*BAL and *Pp*BFD (entry 4), both these enzymes did not accept α-branched aldehydes such as isobutyraldehyde and pivaldehyde. The isovaleraldehyde self-condensation was catalyzed also by the branched-chain 2-ketoacid decarboxylase from *Lactococcus lactis* sup. *cremoris* B1157 (KdcA)[40] although with lower and opposite enantioselectivity (ee 30-47% (*S*)) compared to *Pf*BAL (ee 89% (*R*)) and *Pp*BFD (ee 85% (*R*)). In spite of the physiological 3-methyl-2-oxobutanoic decarboxylase activity, similarly to *Pf*BAL and *Pp*BFD, KdcA did not catalyze the isobutyraldehyde self-carboligation as well.

It has to be mentioned that carboligase activity with linear C3-C6 aliphatic aldehydes as substrates was also observed for the *Ap*PDC-Glu469Gly, although not exploited for preparative purposes.[28]

Ascribable to the aliphatic benzoin-type reaction is also the formal homo-coupling of glycolaldehyde catalyzed by transketolases from *E. coli* (*Ec*TK) using β-hydroxypyruvate as donor (entry 5). The (*S*)-enantiomer (95% ee) of the expected 1,3,4-trihydroxybutane-2-one was obtained in 60% yield.[41] The hydroxypyruvate/glycolaldehyde coupling has been promoted also by PDC from *S.cerevisiae* and by the E1 component of the pyruvate dehydrogenase enzyme system (PDH-E1) from *E. coli*, which afforded the (*S*) and (*R*) enantiomers, respectively; in this study, however, conversion and ee data were not reported.[42] The 1,4-dibenzylated analogue of 1,3,4-trihydroxybutane-2-one has been obtained by the self-condensation of benzyloxyacetaldehyde catalyzed by BAL.[43]

5. Aliphatic cross-benzoin-type reactions

This type of reactions furnish non-symmetric aliphatic (or aromatic-substituted aliphatic) acyloins. Enzymatic approaches for the asymmetric syntheses of such compounds based on the use of ThDP-dependent enzymes are widespread in the literature. In order to give a rational overview of this topic, since a multitude of enzyme/donor/acceptor combinations have been reported to achieve a wide spectrum of mixed aliphatic acyloins, we have divided this section in five parts, each reporting the results of reactions having in common the transfer of one of the acyl anions shown in Figure 2.

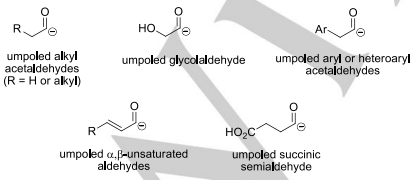
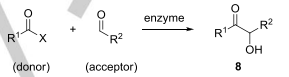


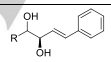
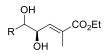
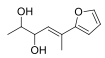
Figure 2. Main types of acyl-anions transferred in ThDP-dependent enzymatic cross-benzoin-type reactions.

5.1. Acyl anion equivalents from alkyl aldehydes

Enzymatic syntheses of unsymmetrically aliphatic acyloins by the ThDP-promoted umpolung of alkyl aldehydes are not so diffused in the literature. Pyruvate and 2-oxobutanoate were condensed with different α,β-unsaturated aldehydes using yeast whole cells (Table 6, entries 1-5).[44] Although because of the simultaneous enzymatic reduction only the diols deriving from the target diketone products could be isolated, this approach demonstrated a good substrate tolerance of yeast PDC. The condensation of pyruvate with C3-C7 linear aliphatic aldehydes have been reported with purified PDCs from *S. cerevisiae*[45] and *Zigosaccharomices bisporus* (entries 6-10).[46] Acyloins were formed with low conversion (2-41%) on an analytical scale and the absolute (*R*)-configuration has been assigned only for the hexanal derivative (ee 69 %, entry 9).[45] Finally, worthy to be mentioned is the addition of the umpoled cyclohexane carboxaldehyde to acetaldehyde catalyzed by *Pp*BFD. The corresponding product 1-(*S*)-cyclohexyl-3-hydroxypropanone was obtained with 21% conversion and 61% ee (entry 11).[18]

Table 6. ThDP-dependent enzyme-catalyzed synthesis of unsymmetrically substituted aliphatic acyloins.

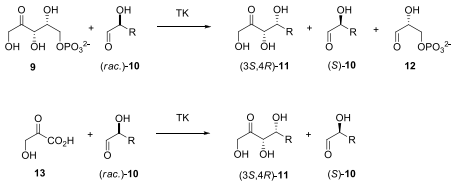


Entry	Product **8**	R	Biocatal.	Conv.(%); ee (%)	Ref.
1		Me	yeast	20 n.d	44
2		Et	yeast	15 n.d.	44
3		Me	yeast	20 100 (*R*)	44
4		Et	yeast	18 n.d.	44
5			yeast	15 n.d.	44
6		Me	*Sc*PDC	41 n.d.	45
7		Et	*Zb*PDC	2 n.d.	46
8		Bu	*Zb*PDC	4 n.d.	46
9		Pr	*Zb*PDC	13 69 (*R*)	46
10		Pent	*Zb*PDC	15 n.d.	46
11			*Pp*BFD	21 61 (*S*)	18

5.2. Acyl anion equivalent from glycolaldehyde

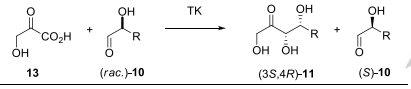
The enzymatic transfer of umpoled glycolaldehyde has been intensively exploited for the synthesis of aliphatic mixed acyloins. Transketolases (Tks) are the elective biocatalysts for such kind of reactions. The natural catalytic activity of

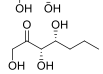
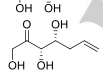
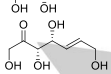
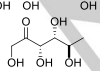
these enzymes is related to the transfer of a C2-ketol unit from D-xylulose-5-phosphate to either D-ribose-5-phosphate or D-erythrose-4-phosphate in the pentose phosphate pathway.[47] The use of hydroxypyruvate **12** in place of the natural ketose donor **7** makes TK-promoted carboligations irreversible through the coupling of the acceptor **8** with the activated glycolaldehyde intermediate, which is formed from **12** with the release of carbon dioxide; by contrast, utilization of **7** as donor generates the aldehyde **11**, which could act as acceptor (Scheme 5).[46]



Scheme 5. Transketolase-catalyzed carboligations with natural ketose and hydroxypyruvate donors.

Table 7. TK-catalyzed cross-couplings of hydroxypyruvate **12** with α-hydroxyaldehydes **8**.

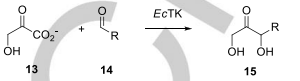


Entry	Product (3*R*,4*S*)-**11**	Yield (%) Tk source	Ref.
1		60 yeast	47a
2		44 yeast	47a
3		45 yeast	47a
4		39 yeast	47a
5		38 yeast	47a
6		30 yeast	47a
7		45 yeast	47a
8		60[a] yeast	47a
9		50[a] spinach	47b

[a] The enzyme was not stereoselective with respect to the configuration of the C3 stereocenter of the acceptor.

The first synthetic uses of TKs were focused on the homologation of α-hydroxylated acceptors **8**. These studies showed that TK either from spinach or yeast are highly stereospecific for (*R*)-aldehydes, generating the new stereocenter of products **9** with (*S*)-configuration (Table 7).[48]

Table 8. Couplings of α-hydroxypyruvate **12** with non-hydroxylated aldehydes **13** catalyzed by wt *Ec*TK and mutant variants thereof.



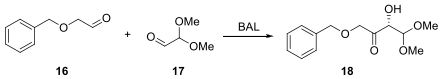
Entry	Product **15**	EcTK variant	Yield (%) Ee (%)	Ref.
1		wt	36; 58 (*S*)	51
		Asp469Glu	70; 90 (*S*)	51
		His26Tyr	63; 88 (*R*)	51
2		wt	36; 75 (*S*)	53a-c
		Asp469Glu	44; 98 (*S*)	53a-c
		His26Tyr	16; 92 (*R*)	53a-c
3		wt	16; 84 (*S*)	53a-c
		Asp469Glu	58; 97 (*S*)	53a-c
		His26Tyr	7; 84 (*R*)	53a-c
4		wt	25; 85 (*S*)	53a-c
		Asp469Glu	47; 97 (*S*)	53a-c
		His26Tyr	12; 84 (*R*)	53a-c
5		wt	7; 74 (*S*)	53a-c
		Asp469Glu	14; 86 (*S*)	53a-c
		His26Tyr	4; 78 (*R*)	53a-c
6		wt	<3; 66 (*S*)	53a-c
		Asp469Glu	18; 86 (*S*)	53a-c
		His26Tyr	21; 83 (*R*)	53a-c
7		wt	<3; rac	53a-c
		Asp469Glu	40; >99 (*S*)	53a-c
		His26Tyr	<3; 30 (*R*)	53a-c
8		wt	<3; rac	53a-c
		Asp469Glu	10; 97 (*S*)	53a-c
		His26Tyr	n.c.; -	53a-c
9		wt	53; 64 (*S*)	53d
		Asp469Glu	59; 87 (*S*)	53d
		His26Tyr	23; 64 (*R*)	53d
10		wt	34; 85 (*S*)	53d
		Asp469Glu	56; 91 (*S*)	53d
		His26Tyr	29; 60 (*R*)	53d
11		wt	5; 93 (*S*)	52a,c
		Asp469Glu	50; 90 (*S*)	52a,c
12		wt	35; 100 (3*S*)[a]	52a,c
		Asp469Glu	30; 100 (3*S*)[a]	52a,c

[a] Diastereomeric ratio (3*S*,4*R*)/(3*S*,4*S*) = 88:12. [b] Diastereomeric ratio (3*S*,4*R*)/(3*S*,4*S*) = 95:5. n.c. (no conversion).

Furthermore, these enzymes[49] as well as TK from *E. coli*,[50] were successfully applied to the condensation of α-

hydroxypyruvate **12** with non-hydroxylated aldehydes **13** (Table 8).In virtue of the moderate stereoselectivity shown by wt-*Ec*TK with propanal (ee 58%), this substrate was used as a probe to evaluate the effects of single-point mutations of active site residues[51] identified by structural and phylogenetic analysis.[52] The Asp469 and the His26 have been recognized as hot spots, whose selective mutation allowed to create *Ec*TK variants with increased (Asp469 variants) or switched (His26 variants) stereoselectivity (Table 8).[53]

Out of the transketolase group, as reported in section 4 (see Table 5, entry 6), BAL catalyzed the addition of the umpoled benzylated glycolaldehyde **16** to various aldehydes.[43] The synthesis of the highly functionalized mixed acyloin **18** of Scheme 6 constitutes a representative example of this strategy.



16 **17** **18**

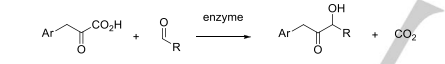
93% *(S)*

Scheme 6. BAL-catalyzed cross-coupling of benzylated glycolaldehyde **16** and 2,2-dimethoxyacetaldehyde **17**.

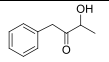
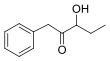
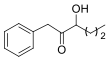
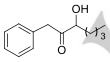
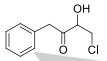
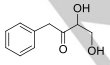
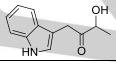
5.3. Acyl anion equivalents from aryl acetaldehydes

Phenylpyruvate is the typical donor used in this kind of enzymatic reaction.

Table 9. Enzymatic couplings of arylpyruvates with aliphatic aldehydes.



Ar = phenyl or 3-indolyl **19**

Entry	Product **19**	Enzyme	Yield (%); Ee (%)	Ref.
1		PhPDC	76; 87	54
		KdcA	49; n.d.	40
2		PhPDC	55; 98	54
3		PhPDC	38; 98	54
4		PhPDC	24; 92	54
5		PhPDC	13; n.d.	54
6		PhPDC	16; n.d.	54
7		PhPDC	19; n.d.	54
		KdcA	23; n.d.	40

n.d. (not detected).

Its enantioselective condensation with C2-C5 linear aliphatic aldehydes, chloroacetaldehyde and glycolaldehyde has been achieved with moderate to good yields and ee ranging from 87 to 98% using phenylpyruvate decarboxylase (PhPDC) from *Achromobacter eurydice* (Table 9, entries 1-6).[54] The acceptor substrate scope resulted to be quite narrow since longer chain aldehydes, aromatic aldehydes, α-methylated, and α,β-unsaturated aldehydes were unreactive or gave negligible conversions. On the other hand, PhPDC resulted to be active also with indole-3-pyruvic acid (entry 7); this donor was reacted with acetaldehyde to give the corresponding acyloin in 19% yield (ee and absolute configuration were not determined).[54] The condensations of phenylpyruvic and indole-3-pyruvic acids with acetaldehyde were also catalyzed by KdcA with 49 and 23% yields, respectively (absolute configuration and ee not determined).[40]

5.4. Acyl anion equivalents from α,β-unsaturated aldehydes

The *Pf*BAL has been used to catalyze the 1,2-addition of various α,β-unsaturated aldehydes to formaldehyde, acetaldehyde or acetaldehyde derivatives as acceptors.[55] The corresponding mixed acyloins **20** have been selectively formed with 23-82% yields and ees ranging from 50 to 98% (Table 10). When determined, the absolute configuration of the chiral products was (*R*). In the same study, the *Pp*BFD was able to catalyze only some of the same reactions affording the (*R*)-products with lower yields and ees respect to *Pf*BAL, with the exception of the derivative of entries 5 that was obtained in 39% yield with the opposite (*S*)-stereochemistry (ee 94%). Moreover, the *Pp*BFD-His281Ala variant, which demonstrated a higher carboligation activity compared to the wild type enzyme in cross-benzoin reactions,[26b,30] did not accept most of the unsaturated aldehydes as substrate.

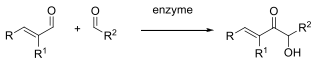
5.5. Acyl anion equivalent from succinic semialdehyde

Three ThDP-dependent enzymes have been adopted to catalyze the 1,2-addition of the umpoled succinic semialdehyde generated from α-ketoglutaric acid **21** to various aliphatic aldehydes. One of these enzymes is the MenD (2-succinyl-5-enolpyruvyl-6-hydroxy-3-cyclohexene-1-carboxylate synthase) from *Escherichia coli* K12, whose physiological activity consists in the decarboxylation of α-ketoglutarate and the subsequent 1,4-addition of the resulting acyl anion equivalent to isochorismate.[56] The other two enzymes are the products of the two homologous genes SucA and Kgd encoding for the 2-oxoacid decarboxylase component (E1) of the ketoglutarate dehydrogenase enzyme system (KGD) in *E.coli* K12[57] and *Mycobacteriun tuberculosis,*[58] respectively.

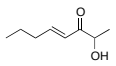
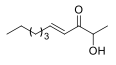
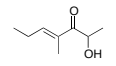
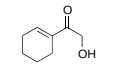
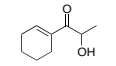
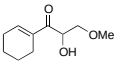
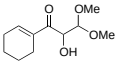
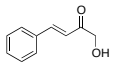
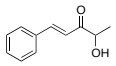
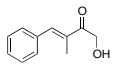
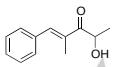
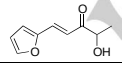
A comparative study on the carboligation activity of these three enzymes has been conducted using the physiological donor **21** and various aliphatic and aromatic aldehydes.[59] All the three enzymes catalyzed the complete conversion of C2-

C6 linear alkyl aldehydes to the corresponding (*R*)-acyloins (Table 11, entries 1-4).

Table 10. Aliphatic cross-benzoin-type reactions with α,β-unsaturated aldehyde donors.



(donor) (acceptor) **20**

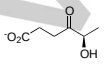
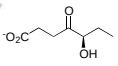
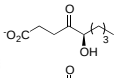
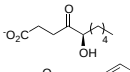
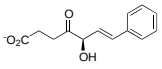
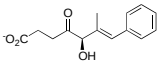
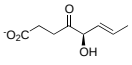
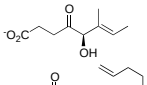
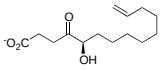
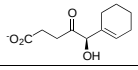
Entry	Product **20**	Enzyme	Yield (%); Ee(%)	Ref.
1		*Pf*BAL	80; 87	55
		wt- PpBFD	79; 24	55
		*Pp*BFD-His281Ala	63; 27	55
2		*Pf*BAL	63; 77	55
		wt- PpBFD	26; 23	55
3		*Pf*BAL	71; >98	55
4		*Pf*BAL	56; - (-)	55
5		*Pf*BAL	23; >98 (*R*)	55
		wt- PpBFD	39; 94 (*S*)	55
6		*Pf*BAL	24; 90 (*R*)	55
7		*Pf*BAL	29; 93 (*R*)	55
8		*Pf*BAL	51; - (-)	55
9		*Pf*BAL	80; 80 (*R*)	55
10		*Pf*BAL	82; - (-)	55
11		*Pf*BAL	75; 96 (*R*)	55
12		*Pf*BAL	48; 50 (*R*)	55

n.d. (not detected).

SucA showed the highest stereoselectivity with ee ranging from 82 to 94%. Moderate to good ee were observed with Kgd (ee 82-70%), whereas MenD showed the lowest enantioselectivity (ee <5-63%). Using α,β-unsaturated aldehyde acceptors (entries 5-8) very poor results were obtained with SucA, whereas Kgd was practically inactive. On the contrary, MenD catalyzed the almost complete conversion of the same substrates. The optical purity was determined only for the 2-methylcinnamaldehyde derivative that resulted practically enantiopure (entry 6). Dec-9-enal and cyclohexene-1-carboxaldehyde were tested only with MenD, which afforded the corresponding products in 60 and 21% yield, respectively (entries 9-10).[37] Worthy of note is that, in spite of the natural Stetter-like physiological activity, MenD exclusively afforded the 1,2 adducts of α,β-unsaturated aldehydes.

Table 11. Aliphatic cross-benzoin-type reactions catalyzed by SucA, Kgd and MenD.

21 **22**

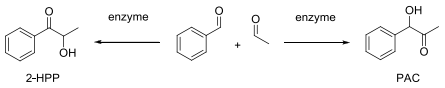
Entry	Product **22**	Yield (%); Ee (%) SucA	Kgd	MenD
1		>99 94	>99 76	>99 <5
2		>99 94	>99 82	>99 11
3		>99 90	>99 70	>99 63
4		>99 82	>99 72	>99 61
5		8 n.d.	n.c .	89 n.d.
6		4 n.d.	n.c.	>99 >99
7		n.c.	n.c.	>99 n.d.
8		12 n.d.	n.c.	98 n.d.
9		-	-	60[c]
10		-	-	21[c]

[a] Determined by ^{1}H-NMR spectroscopy. [b] The absolute configuration for all products was determined as (*R*) on the basis of circular dichroism. [c] Isolated yield. n.d. (not detected); n.c. (no conversion).

6. Aliphatic-aromatic and aromatic-aliphatic cross-benzoin type reactions

Reactions belonging to this category can be divided into two groups depending on the reciprocal roles played by the aliphatic and aromatic reagents: with aliphatic donors and

aromatic acceptors the products are α-oxo alkyl aryl carbinols (aliphatic-aromatic cross-benzoin-type reactions). On the contrary, the combination of aromatic donors with aliphatic acceptors furnishes α-hydroxy alkyl aryl ketones (aromatic-aliphatic cross-benzoin-type reactions). Phenylacetylcarbinol **4** (PAC) and the 2-hydroxypropiophenone (2-HPP) are representative products arising from the above routes (Scheme 7). The enzymatic reactions affording these two compounds and their analogues (PACs, 2-HPPs) have been deeply investigated; therefore, in the following two subsections, we report the results that, in our opinion, represent the more significant advances in the enzymatic synthesis of these two classes of compounds.

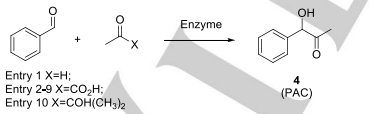


Scheme 7. Enzymatic couplings of benzaldehyde and acetaldehyde producing phenylacetylcarbinol (PAC) and 2-hydroxypropiophenone (2-HPP).

6.1. PACs synthesis

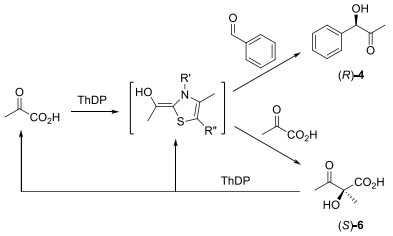
The yeast catalyzed synthesis of (*R*)-PAC was firstly reported in 1921[60] and immediately exploited for the industrial production of (−)-ephedrine.[61] Despite this long time knowledge, the role of PDC in this transformation was demonstrated only at the beginning of the 90's thanks to studies describing the activities of PDCs from different microbial sources.[62] The *in vivo* production of PAC has been observed with many microorganisms,[63] while the more utilized isolated enzymes have been, so far, PDCs from *S. cerevisiae*, *Z. mobilis,* and *A. pasteurianus* (Table 12, entries 1-3).[64] Several PDC variants with improved carboligation activity have been obtained by site-directed mutagenesis. The aforementioned *Zm*PDC-Glu473Gln variant (section 4) catalyzes the PAC formation with a 20-fold increased rate affording a 3-fold higher yield (from 30 to 98%) in comparison with the *wt* enzyme (Table 12, entry 4).[31a]

Table 12. Enzymatic syntheses of phenylacetylcarbinol (PAC) **3**.



Entry	Enzyme	Conv. (%); Ee (%)	Ref.
1	wt-*Ap*PDC	30; 93 (*R*)	64
2	wt-*Zm*PDC	30; 98 (*R*)	31a
3	wt-*Sc*PDC	75; 90 (*R*)	64
4	*Zm*PDC-Glu473Gln	98; 98.4 (*R*)	31a
5	*Zm*PDC-Glu473Phe	93; 99.6 (*R*)	38
6	*Zm*PDC-Glu473Gly	95; 76 (*S*)	38
7	*Ap*PDC-Glu469Gly	95; 70 (*S*)	28
8	*Ec*AHAS-I	75; 90 (*R*)	66
9	CDH	75; 90 (*R*)	69
10	Ao:DCPIP OR	84; 94 (*S*)	71

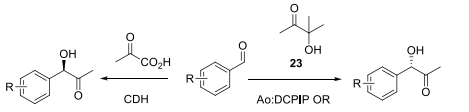
This improved performance results from the stabilization of the enamine/carbanion intermediate, which is protonated 2000-fold slower in the mutant enzyme as a cause of the loss of GLu473 general acid catalysis. Homologous glutamate residues have been recognized in PDCs from *S. cerevisiae*[65] and *A. pasteurianus*[28] at 477 and 469 positions, respectively. As reported in the section dedicated to benzoin reactions, the Glu469 of *Ap*PDC is also the main responsible for the constraint of the active site (*S*)-pocket. The opening of this active site region through the mutation of Glu 469 with Gly, made possible the parallel positioning of the donor and acceptor side chains that is a prerequisite for (*S*)-selectivity. The resulting *Ap*PDC-Glu469Gly variant represents the first example of a PDC suitably tailored for (*S*)-selective synthesis of PAC (Table 12, entry 7).[28] This approach has been extended also to the *Zm*PDC, whose stereoselectivity in the PAC synthesis has been finely tuned through single-point mutations of the Glu473. The authors demonstrated that the substitution of this residue with bulkier side chain amino acids increased the (*R*)-specificity. On the contrary, the exchange with very small residues allowed the production of (*S*)-PAC. The best results were obtained with the *Zm*PDC variants Glu473Phe (ee 99.6% *R*) and Glu473Gly (ee 76% *S*) (Table 12, entries 5 and 6).[38] The enzymatic synthesis of (*R*)-PAC has been carried out also with the acetohydroxyacid synthase (isoenzyme I) from *E. coli* (*Ec*AHAS-I), an enzyme whose physiological carboligase activity consists in the synthesis of (*S*)-acetolactate ((*S*)-**6**) or (*S*)-2-hydroxy-2-ethyl-3-oxobutyrrate through the homo-coupling of pyruvate or the cross-coupling of pyruvate with the 2-ketobutyrate, respectively.[66] The potential double role of pyruvate does not negatively affect the synthesis of (*R*)-PAC mediated by *Ec*AHAS-I. In fact, in the presence of an equimolar amount of benzaldehyde (40 mM), about 90% of the starting pyruvate can be converted to (*R*)-PAC (ee >98%). This is possible because the formed acetolactate is cleaved by the same enzyme with formation of the activated acetaldehyde and pyruvate, which can react with benzaldehyde to give (*R*)-PAC (Scheme 9).[67] This intrinsic carboligation efficiency is the reason of the recent adoption of *Ec*AHAS-I for the totally enzymatic cascade syntheses of (1*R*,2*S*)-norephedrine and (1*R*,2*R*)-norpseudoephedrine.[68]



Scheme 8. Formation and cleavage of acetolactate during the synthesis of (*R*)-PAC catalyzed by EcAHAS-I.

Several other enzymes have been recently added to the enzyme toolbox for the PAC synthesis. The ThDP-dependent enzyme 1,2-cyclohexanedione hydrolase (CDH) is active in the bacterial anaerobic degradation of alicyclic alcohols.[69] Its natural substrate is the 1,2-cyclohexanedione that it is cleaved forming 6-oxohexanoic acid. Other than for the already discussed synthesis of acetoin,[32] the unphysiological carboligation activity of this enzyme has been also recently exploited to produce (*R*)-PAC through the condensation of pyruvate and acetaldehyde. The CDH accepted as substrates a broad range of variously monosubstituted benzaldehydes (Table 13, columns 3 and 4) and sterically hindered aromatic aldehydes (Table 14, values a) giving the corresponding (*R*)-PAC analogues with elevated ee (92-99%).[70]

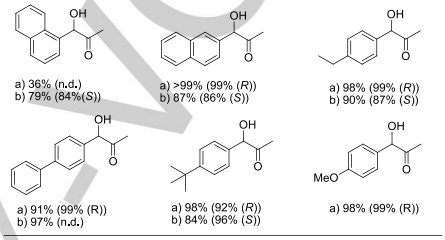
Table 13. Monosubstituted PACs synthesized by CDH and Ao:DCPIPOR



Entry	R	CDH		Ao:DCPIP OR	
		Conv. (%)	Ee (%)	Conv. (%)	Ee (%)
1	2-F	5	n.d.	>99	92
2	3-F	47	98	94	80
3	4-F	68	96	99	67
4	2-Cl	6	n.d.	89	78
5	3-Cl	34	98	>99	78
6	4-Cl	82	96	61	89
7	2-Br	24	99	80	89
8	3-Br	49	96	80	78
9	4-Br	69	95	>99	68
10	2-Me	62	99	>99	97
11	3-Me	91	98	>99	91
12	4-Me	97	96	>99	96
13	2-OH	19	n.d.	94	n.d.
14	3-OH	82	99	99	n.d.
15	4-OH	30	98	52	n.d.
16	2-NO_2	0	-	86	n.d
17	3-NO_2	20	n.d.	91	n.d
18	4-NO_2	5	n.d.	95[a]	-

Worth of note is that, before this study, hydroxyl- and nitro-benzaldehydes had been rarely used with ThDP-dependent enzymes, while the corresponding PACs had been formed by CDH although with moderate conversions (Table 13, entries 13-15, 17 and 18). Very recently, our group demonstrated that also the acetoin:dichlorophenolindophenol oxidoreductase (Ao:DCPIP OR) can be used to catalyze the asymmetric synthesis of PACs.[71] This enzyme is involved, together with the other components of the acetoin dehydrogenase enzyme system (ADH-ES), to the bacterial degradation of acetoin.[72] As for most of the enzymes discussed until this point, also for the Ao:DCPIP OR a side carboligation activity has been reported, whose exploitation for the asymmetric synthesis of tertiary alcohols will be discussed in the next section.[73] Taking advantage of the recently introduced use of methylacetoin **23** as acetyl anion donor, we have expanded the reaction scope of the Ao:DCPIP OR to the very rare synthesis of (*S*)-PACs. The enzyme from *Bacillus licheniformis*, cloned and overexpressed in *E. coli*, has shown an impressive substrate tolerance accepting all the aromatic aldehydes already used with CDH (Table 13, columns 4 and 5; Table 14, values b) and giving even higher yields compared to CDH with the recalcitrant substrates hydroxyl- and nitro-benzaldehydes (Table 13 entries 13-18). But the real novelty highlighted by our work has been the discovery of a (*S*)-specific wild-type enzyme for the PACs synthesis; in fact, the few enzymes reported to be specific for the formation of (*S*)-PAC are engineered variants of (*R*)-specific wt enzymes (Table 12, entries 6 and 7).[28,38]

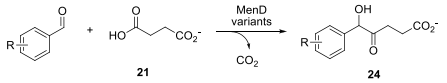
Table 14. Sterically hindered PACs obtained by CDH and Ao:DCPIP OR.[a]



[a] Conditions a): reactions catalyzed by CDH with pyruvate (2.5 equiv.) as donor. Conditions b) reactions catalyzed by Ao:DCPIP OR with methylacetoin **23** (1.5 equiv.) as donor.

The structural diversity of the PAC analogues can also be expanded increasing the complexity of the aliphatic portion. In this perspective, most of the donors used for the aliphatic cross-benzoin-type reactions (section 5) have been also tested with benzaldehyde and benzaldehyde analogs as acceptors. For instance, the enantioselective synthesis of many 5-hydroxy-4-oxo-5-phenylpentanoate derivatives (**24**) have been performed using MenD and α-ketoglutarate as enzyme-substrate pair (Table 15).[74]

Table 15. 5-hydroxy-4-oxo-5-phenylpentanoate derivatives **24** obtained by MenD



Entry	R	wt		Ile474Ala/Phe475Gly	
		Conv. (%)	ee (%)	Conv. (%)	ee (%)
1	H	>99	>99 (*R*)	15	75 (*S*)
2	2-F	>99	94 (*R*)	55	48 (*S*)
3	3-F	>99	96 (*R*)	43	82 (*S*)
4	4-F	>99	99 (*R*)	14	46 (*S*)
5	2-Cl	98	93 (*R*)	57	≤5
6	3-Cl	>99	96 (*R*)	94	89 (*S*)
7	4-Cl	>99	93 (*R*)	15	48 (*R*)
8	3-Br	99	99 (*R*)	87	97 (*S*)
9	3-I	>99	94 (*R*)	86	93 (*S*)
10	3-OMe	99	>99 (*R*)	64	93 (*S*)
11	3,5-OMe	99	98 (*R*)	83	96 (*S*)

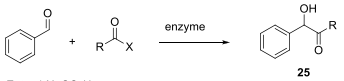
The (*R*)-enantiomers where obtained with almost complete conversions and elevated ees (93->99%) using the wt

enzyme from *E. coli* (Table 15, columns 3 and 4), whereas the double mutated MenD-Ile474Ala/Phe475Gly variant, designed to open the active site according to the "(*S*)-pocket concept", catalyzed the formation of the (*S*)-enantiomers with almost all the same substrates (Table 15, columns 4 and 5).

Several PAC analogues with different aliphatic portions (**25**) have been produced also using the *Ll*KdcA. Although this enzyme was not selective in the acetaldehyde-benzaldehyde coupling affording a 60:40 mixture of (*R*)-2-HPP and (*R*)-PAC (ee 93 and 92%, respectively), it became completely selective when higher aliphatic aldehydes such as propanal, butanal, isovaleraldehyde or cyclopropanecarbaldehyde were used (Table 16, entries 3-6).[40]

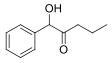
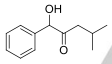
Worth to be mentioned are the few examples of (*S*)-phenylpropionylcarbinol (PPC) synthesis since, until now, this compound represents the only (*S*)-PAC derivative with a different aliphatic portion obtained on preparative scale. In this sense, it is significant the optimization study performed with the ApPDC-Glu469Gly variant, which allowed the production of this derivative (ee 97%) with a conversion >98% and a space-time yield of 17 mM mg^{-1} (Table 16, entry 1).[75] Concerning the PPC production, we have recently proposed a preliminary study where we have demonstrated that, under non-optimized conditions, the combined use of Ao:DCPIP OR as catalyst with 3,4-hexanedione as propionyl anion donor affords (*S*)-PCC (ee 94%) in 60% isolated yield (Table 16, entry 2).[71]

Table 16. Synthesis of PAC analogues form different aliphatic aldehydes.



25

Entry 1 X=CO_2H;
Entry 2 X=$C(O)C_2H_5$;
Entry 3-6 X=H

Entry	Product	enzyme	Yield (%); Ee(%)	Ref.
1		ApPDC-Glu469Gly	>98[a]; 97 (*S*)	75
2		Ao:DCPIP OR	60; 94 (*S*)	71
3		*Ll*KdcA	n.d. >98 (*R*)	40
4		*Ll*KdcA	32; 96,5 (*R*)	40
5		*Ll*KdcA	25; 88 (*R*)	40
6		*Ll*KdcA	14; 98 (*R*)	40

[a] Conversion.

6.2. 2-HPPs synthesis

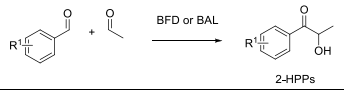
The enzymatic synthesis of 2-HPP (Table 17, entry 1) has been principally addressed with *Pp*BFD and *Pf*BAL, which show a complementary stereoselectivity affording the (*S*)-2-HPP (ee 95%) and the (*R*)-2-HPP (ee 94%), respectively. The carboligation side-activity of BFD, whose physiological role is the cleavage of benzoylformate, was firstly reported in 1992 when, performing the natural reaction in the presence of acetaldehyde (2 equiv.), the authors observed the formation of (*S*)-2-HPP in addition to the expected benzaldehyde.[76] The optimized synthesis with 16 equivalents of acetaldehyde allowed to convert 63% of the starting benzoylformate (100 mM) to (*S*)-2-HPP (ee 92%).[77] As explained in the section 2, in virtue of the reversibility of the benzaldehyde formation step (Scheme 2, step b), benzaldehyde can be engaged in carboligation reactions. It was observed, however, that the yield and the optical purity of the 2-HPP was negatively conditioned by high benzaldehyde concentrations (also because of the side formation of benzoin). These limitations were overcome performing the preparative scale synthesis of (*S*)-2-HPP under continuous-flow conditions. Using a stirred tank enzyme-membrane reactor, a 90% conversion was reached with a 95% ee and a time-space yield of 32 g l^{-1} d^{-1}.[18] In the same work, the donor substrate range was investigated showing that *meta*-substituted benzaldehydes (Table 17, entries 7-11) were the better substrates (68-100% conv., 87-97% ee), while the corresponding *para*-isomers (entries 12-16), gave lower yields (42-85% conv., 82-92% ee). On the other hand, the presence of a substituent in the *ortho* position (entries 2-6) strongly inhibited the enzyme activity, except for 2-fluorobenzaldehyde (entry 4), whose derivative was obtained with a high conversion level (91% conv., 89% ee). On the contrary, the acceptor substrate range of BFD resulted very narrow since negligible or no conversions were observed using propanal, 2-chloro-acetaldehyde, glycolaldehyde, acrolein or propanal as acceptor with benzaldehyde as donor.[18] The substrate rage limitations of the wild-type BFD have been solved through protein engineering approaches. A random mutagenesis study allowed to identify the Leu476 as a hotspot, whose mutation afforded BFD variants with up to five-fold increased carboligase activity.[78] Furthermore, one of these mutants, the BFD-Leu476Gln (Table 17, column 4), resulted more enantioselective than the wt enzyme and it was able to accept *ortho*-substituted benzaldehydes, so allowing the complete conversion of all the tested monosubstituted benzaldehydes to the corresponding 2-HPPs with very high ee (99.5->99%).[79]

To understand the structural basis of the complementary stereoselectivity showed by BAL and BFD in the 2-HPP synthesis, a comparative molecular modeling study of their binding sites have been conducted. It resulted that a small pocket is present in the BFD but not in the BAL acceptor binding site. The acetaldehyde side chain can fit into this pocket and assume an antiparallel orientation with respect to the donor side chain (phenyl). As said before, this donor-acceptor reciprocal orientation has been proposed as the cause of the BFD (*S*)-selectivity, a very rare feature in the field of ThDP-dependent enzyme-catalyzed reactions. This hypothesis was consistent with the (*R*)-selectivity displayed by the same BFD with propanal as donor, whose bulkier side chain cannot enter into the above pocket.[80] This study, which opened the way to the formulation of the already mentioned "(*S*)-pocket concept", also indicated that the acceptor binding site of BFD is predominantly formed by the side chain of

Leu461. As a consequence, this residue has been the target of site-directed mutagenesis experiments that afforded engineered BFD variants (Leu461Ala and Leu461Gly) with an enlarged (*S*)-pocket and, consequently, with the capability to catalyze the (*S*)-selective addition of benzaldehyde to propanal affording 2-hydroxy-1-phenylbutan-1-one **26** in a high enantiopure form (Table 18).[81]

As said above, the *Pf*BAL catalyzes the enantioselective cross-benzoin-type reaction between benzaldehyde and acetaldehyde affording the (*R*)-enantiomer of 2-HPP (Table 17, entry 1, column 5).[19a] The potential of this enzyme for the production of 2-HPP analogues derived from variously substituted benzaldehydes has been explored[19b] and the results illustrated in Table 17 clearly show the opportunity offered by the combined use of the *Pf*BAL and the *Pp*BFD, (as wt enzyme or mutant variants) for the asymmetric synthesis of a wide set of 2-HPP analogues.

Table 17. Synthesis of 2-HPPs catalyzed by BFD (wt and Leu476Gln mutant variant) and BAL (wt).

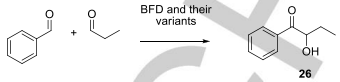


Entry	R[1]	*Pp*BFD wt[a] Conv. (%) ee (%)	*Pp*BFD Leu476Gln[b] Conv. (%) ee (%)	*Pf*BAL Biovar I Wt[c] Conv. (%) ee (%)
1	H	90 95 (*S*)	100 97.5 (*S*)	94 >99 (*R*)
2	2-Me	4 (n.d.)	100 >99 (*S*)	- -
3	2-OMe	0 -	97 >99 (*S*)	63 >99 (*R*)
4	2-F	91 89 (*S*)	100 >99 (*S*)	64 97 (*R*)
5	2-Cl	0 -	100 >99 (*S*)	0 -
6	2-Br	0 -	98 >99 (*S*)	- -
7	3-Me	99 97 (*S*)	100 99 (*S*)	- -
8	3-OMe	94 96 (*S*)	100 99 (*S*)	80 >99 (*R*)
9	3-F	100 87 (*S*)	100 >99 (*S*)	85 95 (*R*)
10	3-Cl	94 94 (*S*)	100 97 (*S*)	94 > 99 (*R*)
11	3-Br	68 96 (*S*)	97 98 (*S*)	88 93 (*R*)
12	4-Me	65 88 (*S*)	100 98 (*S*)	- -
13	4-OMe	23 92 (*S*)	100 >99 (*S*)	64 >99 (*R*)
14	4-F	69 87 (*S*)	100 97 (*S*)	- -
15	4-Cl	85 82 (*S*)	100 96.5 (*S*)	88 >99 (*R*)
16	4-Br	42 83 (*S*)	100 96.5 (*S*)	86 >99 (*R*)

[a] Ref. 18. [b] Ref. 77. [c] Ref.

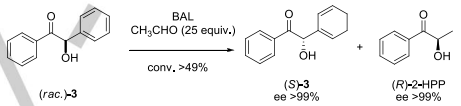
Furthermore, as shown for cross-benzoin reactions, also for the synthesis of 2-HPPs analogues di- and tri-substituted benzaldehydes have been accepted by *Pf*BAL.[19b]

Table 18. Synthesis of 2-hydroxy-1-phenylbutan-1-one **26** with *Pp*BFD (wt and mutant variants)



Enzyme	Yield (%)	Ee (%)
wt-PpBFD	37	21 (*R*)
*Pp*BFD-Leu461Ala	35	93 (*S*)
*Pp*BFD-Leu461Gly	31	97 (*S*)

It is important to emphasize that the combination of benzoin kinetic resolution with the synthesis of 2-HPP catalyzed by *Pf*BAL allowed to prepare both (*S*)-benzoin **3** (ee> 99%) and (*R*)-2-HPP (conv. >49%, ee >99%; Scheme 9).[19a] The same approach has been extended also to the kinetic resolution of mixed-benzoins.[26b]



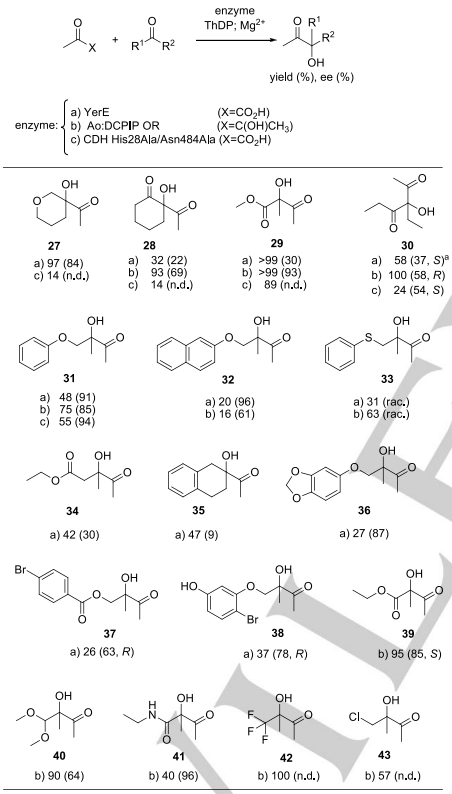
Scheme 9. Synthesis of (2*R*)-HPP and (*S*)-benzoin **3** promoted by BAL through a combined carboligation/kinetic resolution approach.

7. Aldehyde-ketone cross-benzoin-type reactions

In almost all the benzoin-type reactions described until this point, independently from the nature of the donor (aldehyde, α-ketoacid or α-hydroxyacid), the acyl anion equivalent attacks an aldehyde group. As a consequence, secondary alcohols (primary in the case of formaldehyde as acceptor) are produced. In principle, the benzoin-type reaction could be also exploited to produce tertiary alcohols by using ketones as acceptors. In spite of its high synthetic value, this kind of transformation has been only limitedly explored. Tertiary carbinols are generated with a new stereocenter by enzymatic benzoin-type reactions having a α-ketoacid as acceptor: (*S*)-acetolactate **6** and (*S*)-2-hydroxy-2-methyl-3-oxohexanedioic acid are formed by the homo-coupling of pyruvate or the cross-coupling of α-ketoglutarate and pyruvate, respectively, catalyzed by several enzymes like YerE or MenD.[34b] The synthetic value of these reactions is, however, limited by the intrinsic instability of the products that easily undergo spontaneous decarboxylation giving the corresponding racemic secondary α-hydroxyketones. There are only few examples in the literature of enzymatic cross-benzoin-type reactions affording stable tertiary alcohols. The first one has been published in 2010 and describes the use of the ThDP-dependent enzyme YerE to catalyze the cross-coupling of pyruvate with a broad set of ketone acceptors.[35] The feasibility of the aldehyde-ketone cross-coupling was verified by reacting pyruvate, the natural donor, with the

tetrahydro-2*H*-pyran-3-one, an extremely simplified analogue of the YerE natural substrate, the CDP-3,6-di(deoxy)-4-keto-D-glucose. The desired tertiary alcohol **27** (Table **19**) was obtained with a 97% conversion and a good optical purity (ee 84%); the subsequent substrate scope study demonstrated a very broad tolerance of the procedure, which allowed the enantioselective conversion of cyclic and open-chain ketones as well as diketones and α- and β-ketoesters to the corresponding tertiary carbinols (Table 19).

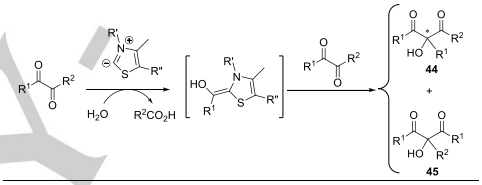
Table 19. Scope of the aldehyde-ketone cross-benzoin-type reaction mediated by YerE, Ao:DCPIP OR and CDH His28Ala/Asn484Ala.



[a] Ref. 82. n.d. (not detected)

Already in 2010, our group reported on the enzymatic aldehyde-ketone cross-benzoin-type reaction, where the same α-diketone served as donor and acceptor substrate. The reactions were catalyzed by an acetoin-inducible enzyme present in the cell free extracts of *B. licheniformis.* The enzyme promoted the ThDP-dependent cleavage of different α-diketones that resulted in the formation of the reactive ylide intermediate (acetyl and propionyl anion equivalents only) and the release of the corresponding carboxylic acid molecule (Table 20).[73a] The subsequent attack of the acyl anion equivalent to one of the two carbonyl groups of the α-diketone generated an achiral product **45** or a mixture of **45** and the chiral product **44**, depending on the structure of the substrate: the symmetric 2,3-buatnedione and 3,4-hexanedione (entries 1 and 2) afforded the achiral products **45** only, while the non-symmetric diketones (entries 3-7) gave mixtures of chiral and achiral derivatives **44** and **45** in different ratios. The optically active products **44**, some of which are known flavoring agents,[73c] showed (*R*)-configurations and ees ranging from 67 to 91%.[73b] The homo-coupling of α-diketones have been also performed under continuous-flow conditions on a packed bed-reactor prepared with the enzyme purified and immobilized onto mesoporous silica.[82]

Table 20. Homo-coupling of α-diketones catalyzed by Ao:DCPIP OR.



Entry	substrate	**44** (Yield, Ee (%))	**45** (Yield, Ee%))
1		-	 (57)
2		-	 (60)
3		 (30, 70 *R*)	 (25)
4		 (42, 67 *R*)	 (19)
5		 (48, 72 *R*)	 (15)
6		 (68, 44 *R*)	 (30)
7		 (45, 76 *R*)	-

We have recently demonstrated that the enzyme responsible for the above reactions, (named acetylacetoin synthase in

references 71 and 80), is the acetoin:dichlorophenolindophenol oxidoreductase (Ao:DCPIP OR), whose natural activity and synthetic exploitation for (*S*)-PACs production have been already illustrated in section 6.1. The known ability of Ao:DCPIP OR to catalyze the cleavage of methylacetoin **23**[83] allowed us to develop a chemoselective synthetic procedure for the asymmetric synthesis of tertiary α-hydroxyketones that was successfully extended to a broad spectrum of substrates.[73d] Several of the products furnished by Ao:DCPIP OR were previously synthesized using YerE as catalyst (Table 19); it was observed that, with open chain ketones, the two enzymes afforded products with opposite stereochemistry (**29**-**32**), while using 1,2-cyclohexanedione as substrate it was obtained the same enantiomer (**28**). Noteworthy, both YerE and Ao:DCPIP OR afforded the thioether **33** as a racemate. In addition to the two wt enzymes YerE and Ao:DCPIP OR, also the cyclohexanedione hydrolase variant CDH-His28Ala/Asn484Ala, which was designed to suppress the natural C-C bond cleavage activities and increase the carboligation potential, was able to catalyze several of the above aldehyde-ketone cross benzoin reactions (products **27**-**31**), showing substrate range and stereoselectivity similar to those of YerE.[82]

8. Conclusions

In the present microreview, we highlighted the opportunities offered by the ThDP-dependent enzymes as catalysts for asymmetric benzoin-type reactions. The selected results herein illustrated demonstrate the synthetic potentials of this class of enzymes, which allow to synthetize a wide variety of optically active α-hydroxyketones using inexpensive reactants and under environmental benign reaction conditions.

Some of the enzymatic benzoin-type reactions discussed in the previous pages, like the synthesis of benzoins catalyzed by BAL and BFD or that of PAC promoted by various PDCs, have been deeply investigated: significant improvements of the enzymes and optimization of the reaction conditions have allowed to reach excellent of levels of conversion and stereoselectivity. Nevertheless, most of the other benzoin-type reactions reported above have not been studied in detail, hence their demonstrated feasibility can be the starting point for furthers investigations. For several reactions, the literature suggests to address further studies on the expansion of the substrate range, while in other cases the optimization of the reaction conditions could be an interesting issue. In addition, the products of some specific enzymatic benzoin-type reactions are well-known synthons, as a consequence these reactions could be exploited in the context of multi-step asymmetric syntheses. In general, every result that contributes to define the substrate-, chemo- and stereoselectivity of a given enzyme can provide information on the active site architecture, which can be used to design engineered variants with modified activity or selectivity.

Finally, a fascinating research issue can be envisaged in the research of not-yet exploited enzymatic carboligation activities. In this sense, the recently introduced benzoin-type reactions catalyzed by YerE, MenD, CDH or Ao:DCPIP OR are representative examples.

Keywords: Asymmetric synthesis; Benzoin reaction; C−C coupling; Enzyme catalysis; Thiamine diphosphate;

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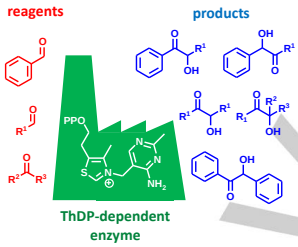
Entry for the Table of Contents (Please choose one layout)

Layout 1:

MICROREVIEW

Thiamine diphosphate (ThDP)-dependent enzymes are very efficient catalysts for asymmetric benzoin-type reactions. Through chemo- and stereoselective coupling of various carbonyl reagents promoted by enzymes of this family a wide variety of optically active α-hydroxyketones have been produced. Herein, an overview of the synthetic relevant enzymatic benzoin-type reactions is given.

*Enzyme Catalysis



Key Topic*

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ThDP-Dependent Enzymes as Catalytic Tools for the Asymmetric Benzoin-Type Reaction